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Cytogenetic and molecular studies of trisomy 13

TERRY HASSOLD*, PATRICIA A JACOBS*, MARK LEPPERT†, AND MICHAEL SHELDON*

*From *the Division of Human Genetics, Department of Pediatrics, Cornell University Medical College, New York, NY 10021; and †the Howard Hughes Medical Institute, University of Utah Medical Center, Salt Lake City, Utah 84132, USA.*

SUMMARY Chromosome heteromorphisms, restriction fragment length polymorphisms, or both were used to study the parental origin of 33 cases of simple trisomy 13 and eight cases of translocation trisomy 13. The most common origin for the simple trisomies was non-disjunction at maternal meiosis I, while for the translocations an equal number of paternally and maternally derived cases was observed.

In seven of the simple trisomies, information was obtained from both the cytogenetic and molecular markers, making it possible to study recombination between the two non-disjoined chromosomes. Five of the seven cases involved errors at meiosis I, with crossing over being detected in two of three cases of maternal origin and in one of two cases of paternal origin. This indicates that absence of recombination because of pairing failure is unlikely to be of major importance in the genesis of trisomy 13.

Almost all information on the parental origin of autosomal trisomy has come from analysis of chromosome heteromorphisms, cytologically detectable variants which occur regularly on 10 of the 22 autosomes. This technique has been most extensively used to investigate the origin of trisomy 21 in liveborns and data are now available on over 1000 families.[1 2] Additionally, there are limited data on liveborn subjects with trisomy 13[3] and on spontaneously aborted conceptuses with trisomies 3, 4, 9, 13, 14, 15, 16, 21, and 22.[4]

Cytogenetic heteromorphisms have provided useful information on the relative importance of different meiotic errors to the genesis of trisomy, but there are several important limitations to the technique. For example, the X chromosome and the majority of the autosomes are not heteromorphic and therefore cannot be studied with this method, and there is relatively little variation in the heteromorphic regions of several other chromosomes (for example, 3, 4, 9, and 16). Furthermore, analysis of the variants involves subjective evaluation of size and staining intensity and, even when the scoring is done blindly and by independent observers, it is possible that a small proportion of parental origin assignments will be wrong.

Some of these difficulties can now be overcome by applying restriction fragment length polymorphism (RFLP) analysis to the study of the origin of trisomy. The advantages of this approach are that (1) the detection of allelic variation using RFLPs is objective; (2) the DNA fragments show gene dosage, a prerequisite for determination of the parental origin of trisomies using a two allele system, and (3) the availability of multiple polymorphisms on each chromosome makes it likely that the parental origin of virtually every trisomy can be unambiguously determined. Preliminary results from studies that have used RFLPs clearly show the efficiency of this method.[5]

Additionally, by combining analysis of chromosome heteromorphisms with analysis of RFLPs, it is now possible to address questions which were previously intractable. One of the most interesting of these is the possibility that aberrant levels of recombination may be important in non-disjunction. In species with chiasmate meiosis, maintenance of pairing in meiosis I is presumably dependent on the presence of at least one chiasma per bivalent.[6] Thus, reduction or elimination of crossing over may lead to univalent formation and ultimately to non-disjunction and there is some evidence from studies of experimental animals[7 8] to suggest that this may be an important source of trisomy. Furthermore, if multiple exchanges impede the orderly separation of bivalents at anaphase I, abnormally high levels of recombination might also be associated with non-disjunction.

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Both of these suggestions can now be directly tested in humans by using centromere mapping techniques to compare the frequency of crossing over in normal meioses and meioses resulting in trisomy. The mapping method using trisomy is essentially the same as that previously described for ovarian teratomas,[9] that is, chromosome heteromorphisms or pericentromeric RFLPs or both are used to determine the meiotic stage of origin of the additional chromosome and centromeric distances of RFLPs estimated by evaluating the level of heterozygosity among trisomic RFLPs for which the parent of origin is known to be heterozygous. In trisomy of meiosis I origin, increasing homozygosity is directly proportional to increasing centromeric distance; in trisomy of meiosis II origin the opposite relationship holds.

Using this approach, Antonarakis *et al*[10] recently studied the inheritance of RFLPs in 34 subjects with Down's syndrome, their parents, and normal sibs. They observed statistically significant reductions in the map distances associated with trisomy 21 and were only rarely able to detect recombination involving the two non-disjoined chromosomes. From this they suggested that defective pairing may be responsible for a large proportion of cases of trisomy 21.

In this report, we present our preliminary observations on centromere mapping in trisomy 13, and the results of our cytogenetic and molecular studies of parental origin in 33 cases of simple trisomy 13 and eight cases of translocation trisomy 13. Our results show the efficiency of combining cytogenetic with molecular analysis to study parental origin, as we were able to specify the parent and meiotic stage of origin in all cases studied in this way. We also show that pairing failure is not the most important cause of trisomy involving chromosome 13, as crossing over between the two non-disjoined chromosomes was detected in almost all cases that were studied.

Materials and methods

ASCERTAINMENT OF CASES

From 1976 to 1985, we conducted a cytogenetic survey of all spontaneous abortions occurring at a single hospital in Honolulu. During the study, we identified 46 abortuses with simple trisomy 13 and 16 abortuses with translocation trisomy 13, and details of these cases are given in the previous paper in this issue.[11] Parental blood samples were obtained in 38 cases. In 31 of these, cytogenetic markers alone were used to evaluate the parental origin of the abnormality. In seven cases DNA was obtained from fetal tissue or cultured cells and from the parental blood samples, making it possible to evaluate both cytogenetic and DNA markers.

Three other cases involved liveborn infants, one of whom was a complete trisomy 13, one a normal/trisomy 13 mosaic, and one had an unbalanced 13;13 translocation. In the latter two cases, the initial cytogenetic diagnosis was made at another laboratory and the case subsequently referred to us because of our interest in parental origin studies.

CYTOGENETIC ANALYSIS

Chromosome heteromorphisms of the parents and trisomic fetuses or infants were compared using Q banding with dichloromethoxyacridine/spermine.[12] All cases were examined directly under the microscope by at least two independent observers, and, in the event of a disagreement, the final decision taken was the most conservative one compatible with both sets of observations.

For each case, conditional probabilities of the observations were assigned using the formulae of Jacobs and Morton,[13] and the combined data then used to calculate maximum likelihood estimates of the different meiotic mechanisms of origin. In all cases, we have assumed that the trisomy resulted from a meiotic and not a mitotic error. Additionally, we have assumed that crossing over did not occur between the centromere and the heteromorphic region,[14] and therefore we can specify the meiotic stage of origin on the basis of the cytogenetic observations alone.

DNA ANALYSIS

DNA from fetal tissue, cultured fetal cells, and peripheral blood samples was prepared as previously described.[15] DNA samples were digested with the appropriate endonucleases using the conditions specified by the manufacturer (International Biotechnologies Inc) and the digested DNA size fractionated overnight on 0·8 to 1·0% agarose gels. The samples were then transferred to nylon membranes (Zetabind, AMF-Cuno Inc) using the method of Southern[16] and, after prehybridisation, hybridised to nick translated ^{32}P labelled probes having specific activities of at least 10^8 cpm/μg.

Seven probes detecting chromosome 13 RFLPs were used, namely p7F12, p7D2, pHU26, p9D11, pHUB8, p1E8, and p9A7. Information on the allelic frequencies and fragment sizes, physical locations of the loci defined by the probes, and linkage relationships among the loci has recently been summarised by Leppert *et al.*[17] Three of the probes (p7D2, pHU26, and p1E8) detect variation with one enzyme, but the remainder are useful with more than one enzyme. Thus, by studying p7F12 and p9D11 with *Taq*I and *Msp*I digested samples, p9A7

with *Hind*III and *Msp*I digested samples, and pHUB8 with *Eco*RI and *Hind*III digested samples, a total of 11 probe/enzyme systems was used in the analysis.

Results

PARENTAL ORIGIN OF TRISOMY

Detailed information on the 33 cases of simple

TABLE 1 *Summary of parental origin data from 31 spontaneous abortions and two liveborns with an additional chromosome 13.*

ID No	*Chromosome constitution*	*Chromosome heteromorphisms*			*Conditional probabilities for*				*Parental origin*
		Father	*Mother*	*Fetus*	*Paternal I*	*Paternal II*	*Maternal I*	*Maternal II*	
Spontaneous abortions									
K431	47,XY,+13	aa	aa	aaa	1	1	1	1	Unknown
K435	47,XY,+13	ab	cd	acd	0	0	1	0	Maternal I
K662	47,XY,+13	ab	cd	acd	0	0	1	0	Maternal I
K687	47,XX,+13	ab	cd	aac	0	1	0	0	Paternal II
K1127	47,XY,+13	ab	cd	abc	1	0	0	0	Paternal I
K1154	47,XX,+13	ab	cd	acc	0	0	0	1	Maternal II
K1331	47,XX,+13	ab	cd	acd	0	0	1	0	Maternal I
K1973	47,XX,+13	ab	aa	aaa	0	1/2	1/2	1/2	Unknown
K2015	47,XX,+13	ab	cc	acc	0	0	1	1	Maternal I or II
K2067	47,XX,+13	aa	bc	abc	0	0	1	0	Maternal I
K2109	47,XY,+13	ab	ac	abc	1/2	0	1/2	0	Unknown
K2157	47,XX,+13	ab	ac	abc	1/2	0	1/2	0	Unknown
K2234	47,XY,+13	—	ab	aab	1	1	1	1	Unknown
K2258	47,XX,+13	ab	cd	acc	0	0	0	1	Maternal II
K2260	47,XY,+13	ab	ac	aac	0	1/4	1/2	0	Unknown
K2528	47,XX,+13	ab	aa	aab	1	0	1/2	1/2	Unknown
K2707	48,XY,+13,+21	ab	cd	acd	0	0	1	0	Maternal I
K2710	47,XY,+13	ab	cc	acc	0	0	1	1	Maternal I or II
K2722	47,XX,+13	ab	ac	abc	1/2	0	1/2	0	Unknown
K2766	47,XY,+13	ab	cd	abc	1	0	0	0	Paternal I
K2788	47,XX,+13	aa	ab	aab	1/2	1/2	1	0	Unknown
K2855	47,XY,+13	aa	bb	abb	0	0	1	1	Maternal I or II
K2857	47,XX,+13	aa	ab	aab	1/2	1/2	1	0	Unknown
K2858	47,XY,+13	ab	cd	acc	0	0	0	1	Maternal II
K3057	47,XX,+13	ab	cd	acd	0	0	1	0	Maternal I
K3177	47,XX,+13	aa	bc	abc	0	0	1	0	Maternal I
K3178	47,XX,+13	ab	cd	acd	0	0	1	0	Maternal I
K3283	47,XX,+13	ab	cd	acd	0	0	1	0	Maternal I
K3325	47,XY,+13	aa	bc	abc	0	0	1	0	Maternal I
K3362	46,X,+13	ab	ac	aab	1/2	0	0	1/4	Paternal I
		aa	bb	aab*	1	1	0	0	
K3392	47,XY,+13	ab	ac	acc	0	0	0	1/2 }	Maternal II
		aa	bb	abb*	0	0	1	1 }	
Liveborns									
G72	47,XY,+13	ab	cd	acd	0	0	1	0	Maternal I
X16	46,XX/47,XX,+13	aa	ab	bb/abb	0	0	0	1 }	Maternal II
		aa	ab	abb*	0	0	1	1 }	

*Results of analysis of DNA polymorphisms.

TABLE 2 *Summary of studies of parental origin in 30 cases of trisomy 13*.*

	Meiotic origin of non-disjunction						
	Paternal I	*Paternal II*	*Paternal Either*	*Maternal I*	*Maternal II*	*Maternal Either*	*Unknown*
No of cases	2	1	0	10	4	3	10
Maximum likelihood estimate for occurrence of error†	0·12	0·04	—	0·68	0·16	—	—
Mean maternal age (SD)	26·5 (12·0)	32	—	32·6 (5·7)	36·5 (7·9)	29·3 (5·9)	27·9 (5·6)
	28·3 (9·1)			32·9 (6·3)			

*Excludes one mosaic trisomy 13 (X16) and two double aneuploids (K2707 and K3362).
†Based on the formulae provided by Jacobs and Morton.[13]

trisomy 13 is given in table 1 and a summary of the parental origin studies on the 30 single, complete trisomies given in table 2. Examples of the use of RFLPs in determining parental origin are provided in fig 1. We were able to determine the origin of the additional chromosome in 20 of the 30 cases, with 17 (85%) being maternal and three (15·0%) being paternal in origin. The single most common mechanism of origin was maternal meiosis I non-disjunction, which accounted for an estimated 68% of cases.

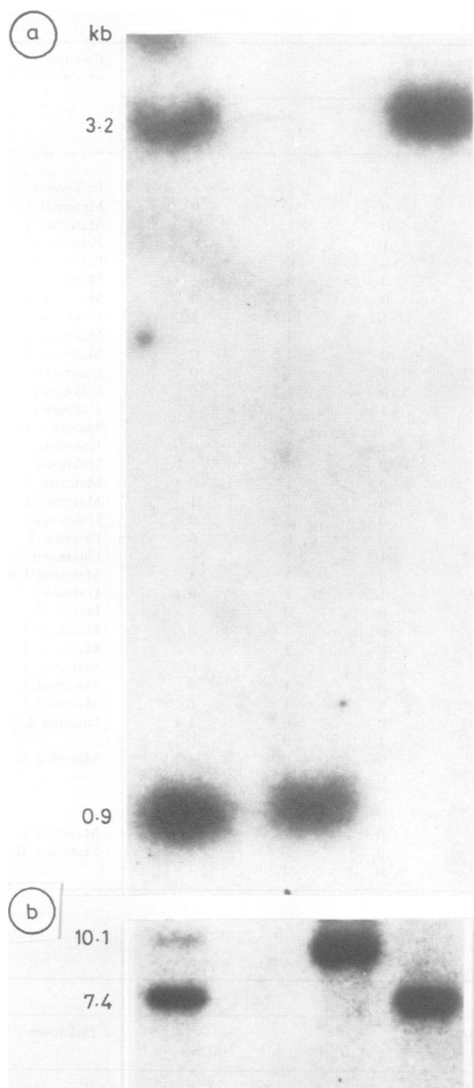

FIG 1 *Use of RFLPs to determine the parental origin of trisomy 13 (in both a and b, lane 1=trisomy, lane 2=father, lane 3=mother). (a) Paternal origin of trisomy. Samples digested with HindIII and probed with p9A7. 1:2 ratio of upper to lower band in this example and in b below is consistent with scanning densitometric readings of normal heterozygotes and trisomies with the opposing genotype. (b) Maternal origin of trisomy. Samples digested with MspI and probed with p1E8.*

Three cases were considered separately as they had more complicated karyotypes. One of these (X16) was a normal/trisomy mosaic with the normal cell line comprising 64·3% (45/70) of all cells examined. Cytogenetic analysis showed that, like most mosaic trisomies,[18] the conception began development as a trisomic zygote, in this case as a result of a maternal meiosis II error. Subsequently the paternal chromosome 13 was lost, presumably at an early division of the zygote, as mosaicism was present in both blood and skin. Thus the 'normal' cell line was, in fact, disomic for a maternal chromosome 13 and had no paternal contribution.

The two remaining cases were both double aneuploids, with K2707 being trisomic for chromosome 21 and K3362 being monosomic for the X as well as being trisomic for 13. In the former case, we were unable to determine the parental origin of the additional chromosome 21, but could not exclude an error at maternal meiosis I (the source of the extra chromosome 13). In the latter case, analysis of X linked RFLPs indicated that the single X was maternal in origin and, by combining the cytogenetic with the molecular results (table 3), the origin of the additional chromosome 13 was determined to be non-disjunction at paternal meiosis I. Thus, in this instance, both fetal chromosome abnormalities involved the paternal complement.

Table 2 also summarises information on maternal age for trisomy of paternal and maternal origin. Although the data are limited, they indicate a strong maternal age component in cases of maternal meiosis I and II origin, but there is no such evidence for the paternally derived cases. This is consistent with the idea that higher maternal age increases the likelihood of non-disjunction and provides no support for the 'relaxed selection' hypothesis,[19] which suggests that the association between trisomy and maternal age derives, at least in part, from decreasing ability to abort an abnormal conceptus with increasing maternal age.

The data on maternal age also provide indirect evidence against the 'production line' hypothesis,[7] which suggests that the effect of maternal age on trisomy results from an increased likelihood of univalency in aged oocytes. If this were the case, maternal age should be raised in trisomy of maternal meiosis I, but not meiosis II, origin. In fact, we observed the highest mean maternal age in trisomy of maternal meiosis II origin. This observation is

TABLE 3 *Analysis of parental origin and recombination in trisomy 13 using chromosome heteromorphisms and RFLPs.*

ID No	*Cytogenetic analysis*	*Locus:* *Probe:* *Enzyme:* *Location:*	*D13S1* *p7F12* *TaqI* *q12→q14*	*D13S1* *p7F12* *MspI* *q12→q14*	*D13S10* *p7D2* *TaqI* *q14*	*D13S7* *pHU26* *BglII* *q22*	*D13S2* *p9D11* *MspI* *q22*	*D13S2* *p9D11* *TaqI* *q22*	*D13S5* *pHUB8* *EcoRI* *q22→qter*	*D13S5* *pHUB8* *HindIII* *q22→qter*	*D13S4* *p1E8* *MspI* *q22→qter*	*D13S3* *p9A7* *HindIII* *q33→qter*	*D13S3* *p9A7* *MspI* *q33→qter*	*Parent and meiotic stage of origin*
K3177	abc*		222†	111†	222	111†	112	222	222†	112	122	112	122	
Fa	aa		22	12	22	11	12	22	12	12	12	12	12	Maternal I
Mo	bc		12	12	22	12	11	22	12	11	22	11	22	
K3283	acd*		222	122‡	222	111	112	222	222	111	122‡	112‡	222	
Fa	ab		22	22	12	11	12	22	22	11	22	11	22	Maternal I
Mo	cd		22	12	22	11	12	22	22	11	12	12	22	
K3325	abc*		222†	222†	222	111	111	122	222†	112	111	111	222	
Fa	aa		12	12	22	11	11	12	22	12	11	12	12	Maternal I
Mo	bc		12	12	22	11	11	22	12	12	11	11	22	
K3392	acc*		112	111‡	222	111	122*	222	222	122†	122*	111	122	
Fa	ab		12	11	22	11	11	22	22	22	11	12	12	Maternal II
Mo	ac		12	12	22	11	22	22	—	12	22	11	22	
X16	abb/bb*		222	222	222	—	222	222	112*‡	111‡	—	—	—	
Fa	aa		12	12	22	—	22	22	22	12	12	12	11	Maternal II
Mo	ab		22	22	22	—	22	22	12	12	22	11	11	
K3362	aab		222	112*	222	111	111	222	112	112	112	122*	112*	
Fa	ab		22	11	22	11	11	22	11	11	12	22	11	Paternal I
Mo	ac		22	22	22	11	12	22	12	12	12	11	22	
K2766	abc*		222	111	222	—	—	222	111†	—	—	—	122	
Fa	ab		22	11	22	11	11	22	12	—	—	—	12	Paternal I
Mo	cd		22	11	22	11	11	22	11	—	—	—	12	
X11	(13;13)+13c*		222	122	222	111	112	222	111	111	112	122*	112*	
Fa	ab		22	12	22	11	12	22	11	11	12	12	12	Paternal
Mo	ac		22	12	22	11	12	22	12	11	11	11	22	

*Results informative for parental origin.

†Results consistent with single (or odd number of) cross overs between centromere and locus, that is, heterozygosity in parent of origin maintained in trisomic in cases of meiosis II origin and reduced in cases of meiosis I origin.

‡Results consistent with no crossing over (or even number of cross overs) between centromere and locus, that is, heterozygosity in parent of origin maintained in trisomic in cases of meiosis I origin and reduced in cases of meiosis II origin.

based on a limited number of cases, but it is substantiated by cytogenetic studies of trisomy 21, which have consistently found the highest maternal ages to be associated with non-disjunction at maternal meiosis II.[2] Thus, it seems likely that maternal age adversely affects chromosome segregation at both meiotic divisions. As recombination occurs only at the first meiotic division, it is doubtful that a deleterious effect of increasing age on recombination is the mechanism responsible for the dramatic increase in non-disjunction with advancing age. Instead, it seems more likely that ageing affects organelles such as the centromere or the spindle, which are common to both meiotic divisions.

PARENTAL ORIGIN OF TRANSLOCATION TRISOMY 13

Table 4 summarises inforr ation on studies of parental origin for seven fetuses and one liveborn infant with de novo translocation trisomy 13. We were able to determine the origin of the translocation chromosome in four of the eight cases, three on the basis of cytogenetic evidence and one on the basis of both RFLPs and chromosome heteromorphisms. In two instances the origin of the abnormality was maternal and in two it was paternal. These results are similar to previous reports of de novo Robertsonian translocations, which have found approximately equal frequencies of paternally and maternally derived translocations.[20]

RECOMBINATION AND NON-DISJUNCTION

Table 3 summarises results on the seven trisomies and one translocation trisomy which were studied using both RFLPs and chromosome heteromorphisms. We were able to determine the parent and meiotic stage of origin of the additional chromosome in all seven trisomies, and therefore in each case were able to study crossing over between the centromere and the loci tested.

Crossing over between the two non-disjoined chromosomes clearly occurred in at least two of three trisomies of maternal meiosis I origin (fig 2). In K3177, heterozygosity in the mother was reduced to homozygosity in the fetus at *D13S1, D13S7,* and *D13S5.* Similarly, in K3325 heterozygosity was reduced at *D13S1* and *D13S5,* with *D13S7* being uninformative. Thus, in both trisomies the most economical explanation of the results is a single exchange between the centromere and the most proximal locus (*D13S1*) with no evidence for further recombination. In the other trisomy of maternal meiosis I origin (K3283) maternal heterozygosity was maintained for all three informative loci (*D13S1, D13S4,* and *D13S3*) and therefore we were unable to document an exchange between the two non-disjoined chromosomes.

We were also able to document crossing over in two of the remaining four trisomies. In K3992, one of the two cases of maternal meiosis II origin, heterozygosity was reduced to homozygosity at *D13S1*, consistent with no crossing over between the centromere and this locus. However, the fetus was heterozygous for the *Hind*III polymorphism at *D13S5,* indicating that a cross over had occurred between *D13S1* and *D13S5*. In K2766, we identified a cross over between the centromere and *D13S5,* the only locus for which the parental RFLPs were potentially informative. We were unable to identify recombinants in the other two cases, but in one (X16) only one locus was potentially informative and in the other (K3362) there were no informative loci.

In the translocation trisomy 13 (X11), analysis of both cytogenetic and molecular markers indicated a paternal origin for the abnormality. Additionally,

TABLE 4 *Summary of data on parental origin from seven spontaneous abortions and one liveborn infant with de novo translocation trisomy 13.*

ID No	*Chromosome constitution*	*Chromosome heteromorphisms*			*Parental origin*
		Father	*Mother*	*Fetus**	
Spontaneous abortions					
K254	46,XY,−14,+t(13;14)	ab	aa	13/14+13b	Maternal
K340	46,XY,−13,+t(13;13)	ab	ac	13/13+13c	Paternal
K942	46,XY,−14,+t(13;14)	ab	aa	13/14+13b	Maternal
K1398	46,XY,−14,+t(13;14)	aa	aa	13/14+13a	Unknown
K1676	46,XX,−13,+t(13;13)	ab	ab	13/13+13a	Unknown
K2275	46,XX,−13,+t(13;13)	ab	ab	13/13+13a	Unknown
K2571	46,XY,−14,+t(13;14)	ab	ab	13/14+13a	Unknown
Liveborns					
X11	46,XX,−13,+t(13;13)	aa	ab	13/14+13b	Paternal
		ab	aa	abb†	

*Fetus has inherited a translocation chromosome (13;13 or 13;14) and a normal chromosome, designated 13a, 13b, or 13c. When the parental origin of the normal chromosome is known, it is inferred that the error occurred in the other parent.
†Results of analysis of DNA polymorphisms.

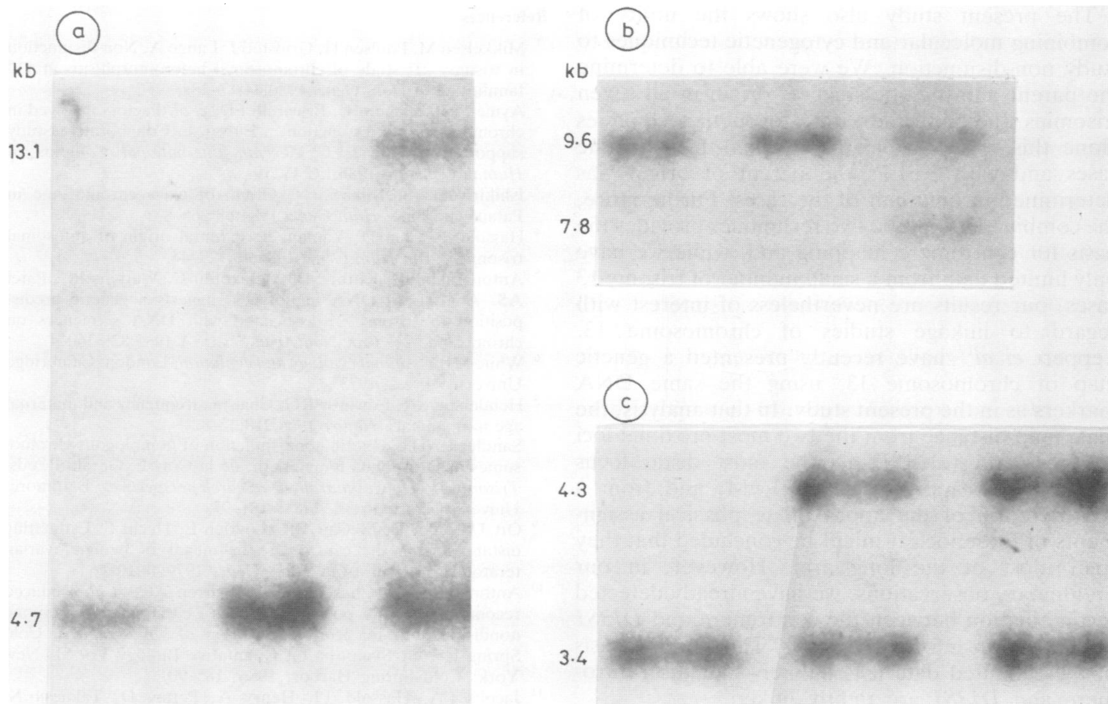

FIG 2 *Examples of the use of RFLPs to identify crossing over between the centromere and a marker locus on chromosome 13. In each case, the origin of trisomy was known to be maternal meiosis I and in each case heterozygosity in the mother is reduced to homozygosity in the trisomic offspring (in a, b, and c, lane 1=trisomy, lane 2=father, lane 3=mother). (a) DNA samples digested with EcoRI and probed with pHUB8. (b) DNA samples digested with BglII and probed with pHU26. (c) DNA samples digested with MspI and probed with p7F12.*

analysis of RFLPs in the trisomic infant showed that crossing over must have occurred between the two paternal homologues, since at *D13S4* paternal heterozygosity was retained and at *D13S3* heterozygosity was reduced to homozygosity.

Discussion

The results of the present study show that non-disjunction at maternal meiosis I is the most common cause of trisomy 13. This is consistent with observations of a maternal age effect in trisomy 13,[21] with results of previous parental origin studies of trisomy 13,[3] and with similar studies of other autosomal trisomies.[4] Additionally, the present likelihood estimates of different meiotic errors are remarkably similar to data already available on trisomy 21, the only other autosomal trisomy that has been extensively studied. Thus, we estimate the proportion of trisomy 13 resulting from paternal meiosis I and II and maternal meiosis I and II to be 0·12, 0·04, 0·68, and 0·16 respectively, compared with estimates of 0·13, 0·07, 0·68, and 0·13 for trisomy 21.[4]

These estimates agree well with our previous analysis of a smaller series of trisomies, in which we observed a significantly increased proportion of paternal non-disjunction in trisomies 13 and 21 by comparison with all other trisomies.[4] However, in that report we also considered data on origin obtained from other laboratories and, as the total data set showed no significant difference between trisomy 13 and other trisomies, we suggested that chromosome 21 might be unique in its susceptibility to paternal non-disjunction. That suggestion now seems unlikely to be correct, based on the results of the larger series of trisomy 13 abortuses summarised in the present report. Nevertheless, our analyses continue to support the general idea of variability in frequency of paternal non-disjunction among trisomies, as the estimated level of paternal non-disjunction remains at approximately 5% for trisomies 22 and 16, the two trisomies for which we have the most information (Hassold and Jacobs, unpublished data).

The present study also shows the utility of combining molecular and cytogenetic techniques to study non-disjunction. We were able to determine the parent and meiotic stage of origin in all seven trisomies studied, while with cytogenetic techniques alone this was possible in only two-thirds of the cases and with RFLPs the parent of origin was determined in only half of the cases. Furthermore, the combined use of the two techniques provides the basis for centromere mapping and, while we have only limited data from a small number of trisomy 13 cases, our results are nevertheless of interest with regard to linkage studies of chromosome 13. Leppert *et al*[17] have recently presented a genetic map of chromosome 13, using the same DNA markers as in the present study. In that analysis, the male map distance from the two most proximal loci (*D13S6*) and (*D13S1*) to the most distal locus (*D13S5*) was approximately 70 cM, and from a consideration of the approximate physical assignments of these loci, it might be concluded that they span most of the long arm. However, in our preliminary observations, we have already detected recombination between the centromere and *D13S1* in two of four informative cases. Thus, on the basis of these limited data it is unlikely that the centromere and *D13S1* are tightly linked.

Additionally, our results are of considerable interest in view of the purported association between reduced recombination in maternal meiosis I and trisomy 21.[10] Specifically, we have evidence for recombination in two of the three cases in which the extra chromosome originated in maternal meiosis I, as well as in the single paternal meiosis I case where information on recombination was obtained. These are preliminary observations but, nevertheless, they clearly show that failure to pair is not the only mechanism responsible for non-disjunction in man. However, it is still possible that a proportion of the cases are the result of asynapsis (for example, in K3283 recombinants were not observed in three opportunities), or that a proportion are related to decreased or increased levels of recombination. A much more extensive series of cases, including trisomies with different parental and meiotic origins and involving parents in different age categories, must be examined before the possible role of recombination in the genesis of human trisomy is fully understood.

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Correspondence and requests for reprints to Dr P A Jacobs, Division of Human Genetics, Department of Pediatrics, Cornell University Medical College, 515 East 71st Street, New York, NY 10021, USA.